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Sampling Designs for Estimating the Total Number of Fish in Small Streams

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Abstract

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A common objective of fisheries research is estimating the total number of fish in small streams. The conventional approach involves (1) selecting a small sample of equal-length sections of stream, and (2) estimating the total number of fish in each section using removal method or mark-recapture estimators. Error of estimation of the total number of fish in a stream arises from two sources: (1) extrapolation from the small number of sampled sections to the entire stream, and (2) errors of estimation of fish numbers within sampled sections. This report shows that errors arising from the first source will usually be far larger than those arising from the second source. Total errors of estimation can be reduced by making sampled sections equivalent to natural habitat units. Entire pools or riffles should be sampled rather than fixed-length sections of streams. The relative performances of three alternative sampling designs, which can be used when sampled sections are equivalent to natural habitat units, are contrasted in terms of accuracy and cost-effectiveness. Accuracy of estimation can be dramatically improved if sampling designs account for the usually strong, positive correlation between fish numbers and habitat unit sizes.

Keywords: Sampling designs, population sampling, fish population, fish habitat.

Summary

The traditional sampling design used to estimate the total number of fish in small streams involves (1) dividing a stream into sections of equal length, (2) selecting a simple random sample from these sections, and (3) using some population estimator within each of the selected sections. In sampling theory jargon, this design is termed a two-stage sampling design with equal-sized primary units. Errors of estimation in a two-stage design arise from two sources: (1) errors of extrapolation from the small number of sampled sections to the entire stream, and (2) errors of estimation of fish numbers within sampled sections. Error arising from the first source is measured by the variation among (estimated) primary unit totals and is termed first-stage variance. Error arising from the second source is measured by the average error of population estimation within selected sections and is termed second-stage variance. In the usual small-stream context, first-stage variance is very large compared to second-stage variance.

Large first-stage variance for the traditional design results from equal-sized primary units being of unequal habitat quality. Certain selected sections may consist primarily of riffle habitat, whereas others may consist primarily of pool habitat. Because densities of fish usually vary considerably among habitat types, the equal-sized primary unit design results in substantial variation among the numbers of fish in primary units and, thus, in large first-stage variance.

Stratification can be used to improve the precision of estimation of the total number of fish in small streams. A stream can be mapped and strata formed on the basis of habitat unit types; for example, pools or riffles. Independent samples can then be drawn from each constructed stratum, and independent estimates of the total number of fish within each stratum can be made. An estimate of the total number of fish in the entire stream can then be obtained by summing all stratum estimates; estimated variance of this estimated total can be obtained by summing variance estimates for independently estimated stratum totals.

If stratification is used to improve precision of estimates, then the natural habitat units become the primary sampling units. Thus, a two-stage sampling design appropriate for unequal-sized primary units must be used within each habitat stratum. Three alternative two-stage sampling designs, appropriate when primary units are of unequal sizes, are presented in this report. Two of these designs can substantially improve precision and accuracy of estimation when (1) the range in primary unit sizes is large (> four-fold), and (2) the correlation between primary unit totals and primary unit sizes is large ($r>0.5$). Improved accuracy of both designs is achieved through a significant reduction in first-stage variance as compared to designs that fail to account for the correlation between fish numbers and habitat unit sizes.

Adoption of two-stage sampling designs based on unequal-sized primary units also gives important biological improvements. It is possible to quantitatively study the relationships between fish numbers and the sizes and qualities of habitat units. This kind of information is critical for understanding the dynamics of the abundance of fish in small streams.

Contents

Introduction

Fisheries biologists are often required to provide estimates of the total number of fish in small streams. The purposes for which these estimates are made will influence the required accuracy of estimates and the costs of obtaining estimates. For purposes of crude inventory work, estimates that are within ± 50 percent of the true quantity (with 95 percent probability) may be adequate. But for research purposes, estimates may have to be within ± 10 percent of the true quantity (see Robson and Regier 1964). For example, evaluation of the impacts and cost-effectiveness of current efforts at rehabilitation or enhancement of anadromous salmonid populations will surely require estimates of fish abundance that are within ± 10-20 percent of the true abundance. Have rehabilitation measures actually increased the number of adult fish spawning in streams? Have the benefits of rehabilitation exceeded the costs? Answers to such questions can be provided only through comparison of estimates of abundance before and after rehabilitation. If errors of estimation of abundance are large, then it will be difficult or impossible to detect changes in abundance and to evaluate rehabilitation programs.

Development of schemes designed to reduce errors of estimation of fish abundance requires knowledge of both population estimators and sampling theory. Most fisheries biologists have received training in the application of population estimators, and this report assumes that readers are familiar with standard mark-recapture and removal method estimators (see Seber 1982 for a thorough review of population estimation methods; see Everhart and Youngs 1981, chapter 6, for a brief review). These population estimators are used widely and effectively in small streams. Knowledge of how to apply these population estimators, however, is inadequate for estimating fish abundance. Usually only a very small fraction of a stream can be sampled. How and where to select sample sections of stream must be decided before population estimators can be used within selected stream sections.

Sampling theory concerns itself with methods that determine how and where to select samples, and with how those methods can influence the quality of inferences drawn from samples. The purpose of this report is to illustrate that basic sampling theory principles can be used to develop cost-effective programs for accurate estimation of fish abundance. In most cases, these principles are simple and intuitive; in other cases they are more complex, but still have strong intuitive appeal. Because few fisheries biologists have studied sampling theory, the intuitive basis of these principles will be stressed and simple numerical examples, rather than formal mathematical developments, will be used for illustrative purposes. The initial material on basic sampling theory concepts is provided for the benefit of readers with essentially no sampling theory background and may be skipped by some readers. **Boldface** type is used throughout this report to indicate the introduction of important sampling theory concepts or terms.

Although the bulk of this report is devoted to examination of alternative sampling designs for estimation of the total number of fish, a fisheries biologist may often want to estimate the total biomass of fish. Appendix 4 is devoted to this topic. Readers interested in a more formal presentation of the alternative sampling designs presented in this report are referred to Hankin (1984) and to references cited in that paper.

Basic Sampling Theory Concepts

Expected Value, Variance, and Mean Square Error

An **estimator,** when applied in practice, can result in many possible **estimates.** For example, if a certain number of fish were marked and then released in a section of stream, many estimates of population size would be possible based on distinctive recapture samples. Due to chance, the fraction of marked fish in a given sample may differ from the true marked fraction in the total population; this will cause a population estimator to generate many different sample-based estimates of population size. The quality of an estimator is therefore assessed on the basis of its overall average performance. In the case of a mark-recapture estimator, this overall average performance may be conceptualized by imagining (1) releasing M fish into a population of size Y, drawing a recapture sample of size C, and estimating population size based on the marked fraction in C; and (2) repeating this same experiment an infinite number of times. If the relative frequency of particular estimates of population size were then plotted against estimated population size, a characteristic distribution would result. This distribution is known as the **sampling distribution** of the population estimator and may be characterized, in part, by its mean and variance. In addition, however, one also characterizes the relationship of this distribution to the true population size that the estimator is designed to estimate.

The quality of an estimator is judged on the basis of its sampling distribution by three principal criteria:

1. **Bias**—the average departure of estimates from the true quantity being estimated.
2. **Variance**—the average (squared) variation of estimates from the average of all estimates.
3. **Mean square error**—bias (squared) plus variance.

The statistical meaning of bias is much the same as its meaning in everyday language; variance and mean square error can also be defined in everyday terms that help clarify their meanings. **Precision** is the reciprocal of variance. Thus, if there is a great deal of variation among possible estimates (variance is large), then an estimator has low precision; if variance is small, then an estimator has high precision. But because variance measures only variation among the possible estimates (from the mean of all estimates), precision is not, by itself, a satisfactory measure of estimator performance. Mean square error is a measure of the **accuracy** of an estimator and is defined as the averaged (squared) variation between estimates and the true quantity being estimated. An estimator with small mean square error has high accuracy, whereas an estimator with large mean square error has low accuracy. Note that low accuracy can result from (1) high bias and high precision, (2) low bias and low precision, or (3) some intermediate combination of bias and precision.

An intuitive understanding of the concepts of bias, variance, and mean square error can be conveyed through a bullseye analogy. Consider each of the four diagrams in figure 1 as patterns of darts thrown by contestants in a contest at a local tavern. Figure 1A depicts the pattern of a highly skilled dart thrower. It is tightly packed (small variance) and centers about the bullseye (the quantity to be "estimated"). The pattern has (1) low bias, (2) high precision, and (3) high accuracy. Figure 1B shows the pattern of a rival dart thrower who is also highly skilled, but is using a new set of darts and has yet to adjust for the unfamiliar balance of the new darts. His pattern is highly precise but, because it is biased (that is, off target), it is less accurate than the pattern in figure 1A. Figures 1C and 1D can be thought of as dart patterns for these same two individuals after each has consumed two pitchers of beer. The first individual's pattern (fig. 1C) remains unbiased (it still centers about the bullseye), but it is now extremely imprecise

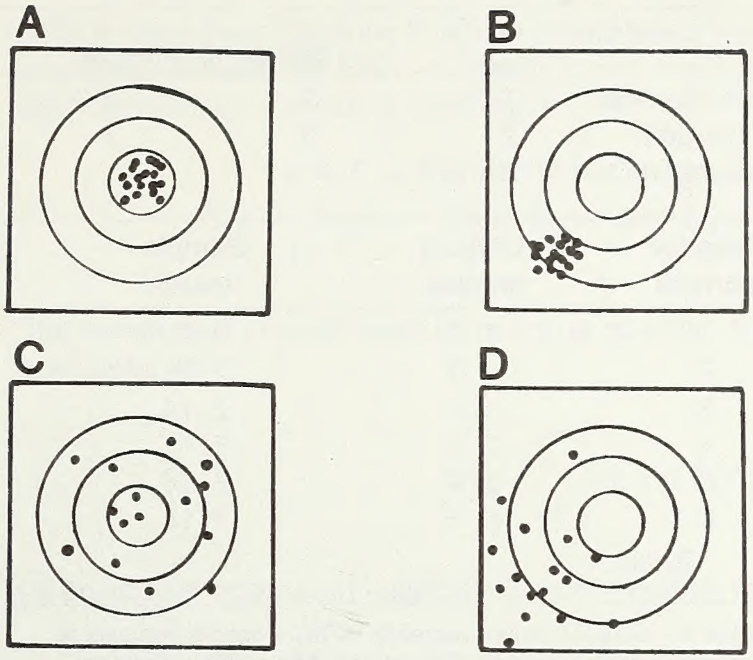

Figure 1.—The bullseye analogy. Various patterns of darts at a target: **(A)** high precision, low bias, high accuracy; **(B)** high precision, high bias, medium accuracy; **(C)** low precision, low bias, low accuracy; and **(D)** low precision, high bias, lowest accuracy.

and, as a result, extremely inaccurate. Figure 1D shows that the second individual's dart pattern retains its bias, is far less precise than in figure 1B, and is even less accurate than the first inebriated dart thrower's pattern. The analogy between dart patterns and the sampling distribution of an estimator illustrates the concepts of bias, precision, and accuracy in an effective conceptual manner and helps prepare one for the quantitive treatment that follows.

Most of sampling theory concerns itself with situations in which there are only a **finite** number of possible samples that can be drawn from a **sampling universe** of finite size. A sampling universe consists of the total number of units from which samples are drawn and the **attributes** of these units. Units may have many attributes. For example, if all pools in a stream constituted the units of a sampling universe, then units could have attributes such as number of fish, area, volume, and average and maximum depth. The objective of sampling is to estimate some collective attribute of the sampling universe on the basis of a small number of units (that is, from a sample). Examples of collective attributes of the pool sampling universe include total area of all pools, total number of fish in all pools, and mean number of fish per pool.

Associated with each of the possible distinct samples of size n units that can be drawn from a sampling universe of size N units, there is an associated probability of drawing that sample. This probability will depend on the selection method used to draw the sample. In the simplest case, a sample is drawn by simple random sampling (SRS); by this selection method no unit can appear more than once in the sample (a **without-replacement** method), and all possible samples are equally likely.

	Sampling universe			
Unit number:	1	2	3	4
Fish/unit:	2	5	7	14
Mean number of fish/unit = 7 = μ				

Possible sample	Units in sample	Sample values	Sample mean ($\bar{y}_t$)	$(\bar{y}_t - \mu)^2$
1	1, 2	2, 5	3.5	12.25
2	1, 3	2, 7	4.5	6.25
3	1, 4	2, 14	8.0	1.00
4	2, 3	5, 7	6.0	1.00
5	2, 4	5, 14	9.5	6.25
6	3, 4	7, 14	10.5	12.25
Totals			42.0	39.00

Example 1.—Simple random sampling (SRS). Possible samples of size 2 selected from a sampling universe of size 4; units in samples; unit values in samples; sample means; and squared deviations of sample means from true mean, $(\bar{y}_t - \mu)^2$.

Example 1 quantitatively illustrates the concepts of expected value and variance when samples of size n = 2 pools are drawn from a sampling universe of size N = 4 pools by SRS. The universe attribute of interest is the mean number of fish per pool ($\mu = 7$). There are six possible simple random samples and each has probability of one-sixth. The **expected value** of an estimator is denoted by $E(\hat{\Theta})$, where Θ is the quantity or attribute of interest, and the "carat" or "hat" (over Θ) indicates an estimator of Θ. Expected value is calculated as:

$$E(\hat{\Theta}) = \sum_{t=1}^{T} \hat{\Theta}_t P_t ;$$

where: T = total number of possible samples; t = 1, 2, . . . , T;
$\hat{\Theta}_t$ = the particular estimate of Θ generated from the t^{th} sample; and
P_t = probability of selecting the t^{th} sample.

In example 1, the estimator of the true mean number of fish per pool is denoted (as a notational convention) by $\bar{y}$, rather than $\hat{\mu}$, and allows estimation of μ from a sample (here of size n = 2 pools):

$$\bar{y} = \sum_{i=1}^{n} y_i/n ;$$

where y_i = the number of fish in pool i, and the summation is over those units that appear in a sample.

SRS is often used because it results in **unbiased** estimators. An unbiased estimator is an estimator such that $E(\hat{\Theta}) = \Theta$; that is, the average value of all possible estimates is the true value of interest. In example 1:

$$E(\bar{y}) = \sum_{t=1}^{T} \bar{y}_t P_t = \sum_{t=1}^{6} \bar{y}_t (1/6) = (1/6) \sum_{t=1}^{6} \bar{y}_t = (1/6) \cdot 42 = 7 = \mu .$$

The variance of an estimator, $\hat{\Theta}$, of a true quantity, Θ, is denoted by $V(\hat{\Theta})$ and is calculated as:

$$V(\hat{\Theta}) = \sum_{t=1}^{T} [\hat{\Theta}_t - E(\hat{\Theta})]^2 P_t \cdot$$

Mean square error of an estimator $\hat{\Theta}$ is denoted by MSE $(\hat{\Theta})$ and is calculated as:

$$MSE(\hat{\Theta}) = \sum_{t=1}^{T} (\hat{\Theta}_t - \Theta)^2 P_t = V(\hat{\Theta}) + [BIAS(\hat{\Theta})]^2 .$$

For an unbiased estimator, $E(\hat{\Theta}) = \Theta$, so that $V(\hat{\Theta}) = MSE(\hat{\Theta})$. For a biased estimator, $E(\hat{\Theta}) = \Theta$ and $BIAS(\hat{\Theta}) = E(\hat{\Theta}) - \Theta$ (which may be positive or negative). Thus, the accuracy of a biased estimator must be measured by $MSE(\hat{\Theta})$; accuracy of an unbiased estimator can be measured simply by $V(\hat{\Theta})$.

In example 1, the variance of $\bar{y}$ can be calculated as:

$$V(\bar{y}) = \sum_{t=1}^{T} [\bar{y}_t - E(\bar{y})]^2 P_t = \sum_{t=1}^{T} (\bar{y}_t - \mu)^2 P_t$$

$$= \sum_{t=1}^{6} (\bar{y}_t - 7)^2 (1/6) = (1/6) \cdot 39 = 6.5 .$$

$MSE(\bar{y})$ would be the same as $V(\bar{y})$ for example 1 because $E(\bar{y}) = \mu$.

Stratification and Relative Efficiency

In many cases, the precision of estimators can be improved by **stratification.** Stratification consists of breaking a sampling universe into two or more groups of units (**strata**) and then drawing **independent** samples from each stratum. The objective of stratification is to group similar units in their own stratum so that variation within constructed strata is small compared to variation between strata. For example, if units were riffles and pools in a stream, then it would make sense to separate units into habitat type strata and to draw independent samples from within each habitat type stratum. This stratification would be effective because densities of fish would usually be different in pools than in riffles. Variation of densities of fish (on a per unit area or volume basis) would be smaller within the pool and riffle strata than variation in densities of fish between pools and riffles.

		Stratum I	Stratum II	
Units in stratum:		1, 2	3, 4	
Fish/unit:		2, 5	7, 14	

Possible sample	Sample units	Sample values	Sample Mean ($\bar{y}_t$)	$(\bar{y}_t - \mu)^2$
1	1, 3	2, 7	4.5	6.25
2	1, 4	2, 14	8.0	1.00
3	2, 3	5, 7	6.0	1.00
4	2, 4	5, 14	9.5	6.25
Totals			28.0	14.50

Example 2.—Stratified random sampling. One possible stratification of the sampling universe presented in example 1. Possible stratified random samples of size 2 (1 unit selected from each stratum); units in samples; unit values in samples; sample means; and squared deviations of sample means from the true mean, $(\bar{y}_t - \mu)^2$.

Example 2 shows one possible stratification of the sampling universe presented in example 1. Two strata have been formed: stratum I contains the two pools that have fewer fish; stratum II contains the two pools that have more fish. If one were to draw a single pool from each of the two strata, and then estimate the mean number of fish per pool, one would be using **stratified random sampling.** Stratified random sampling thus involves selection of units by simple random sampling within each constructed stratum. In contrast, in SRS (without stratification) units are selected by simple random sampling from all of the units in the sampling universe.

When the number of units within each stratum is the same (stratum sizes are equal), the stratified estimator for the mean number of fish per pool is the same as that for SRS (without stratification):

$$\bar{y}_{st} = \sum_{i=1}^{n} y_i/n \, .$$

The nature of the selection method has reduced, however, the number of possible samples that could be drawn and eliminated the possibility of drawing samples that contained the units (1, 2), or (3,4). These samples made the largest contribution to variance in example 1. In example 2, there are only four possible distinct samples; each sample is equally likely and has probability of one-fourth. The expected value of the stratified estimator is also unbiased:

$$E(\bar{y}_{st}) = \sum_{t=1}^{T} \bar{y}_t P_t = \sum_{t=1}^{4} \bar{y}_t (1/4) = (1/4)\cdot 28 = 7 = \mu \, .$$

And the variance of the stratified estimator is substantially less than for SRS (without stratification):

$$V(\bar{y}_{st}) = \sum_{t=1}^{T} [\bar{y}_t - E(\bar{y}_{st})]^2 P_t = \sum_{t=1}^{T} (\bar{y}_t - \mu)^2 P_t$$

$$= \sum_{t=1}^{4} (\bar{y}_t - 7)^2 \cdot (1/4) = (1/4) \cdot 14.50 = 3.625 .$$

As example 3 illustrates, a poor statification can lead to a less precise estimator than SRS (without stratification). In example 3, the stratified estimator is again unbiased, but $V(\bar{y}_{st}) = 11.75 > V(\bar{y}_{srs}) = 6.50$. The stratification in example 3 performed poorly because the most dissimilar units (that had 2 and 14 fish) were grouped in the same stratum. There is thus no assurance that stratification will improve the precision of estimators. Stratified random sampling will be more precise than SRS when the average variation within strata is less than the average variation between strata.

	Stratum I	Stratum II
Units in stratum:	1, 4	2, 3
Fish/unit:	2, 14	5, 7

Possible sample	Sample units	Sample values	Sample Mean ($\bar{y}_t$)	$(\bar{y}_t - \mu)^2$
1	1, 2	2, 5	3.5	12.25
2	1, 3	2, 7	4.5	6.25
3	4, 2	14, 5	9.5	6.25
4	4, 3	14, 7	10.5	12.25
Totals			28.0	37.00

$$E(\bar{y}_{st}) = \sum_{t=1}^{4} \bar{y}_t P_t = (1/4) \cdot 28 = 7 = \mu$$

$$V(\bar{y}_{st}) = \sum_{t=1}^{4} (\bar{y}_t - \mu)^2 P_t = (1/4) \cdot 37.00 = 9.25$$

Example 3.—An alternative stratification of the sampling universe presented in example 1. Possible stratified random samples of size 2 (1 unit selected from each stratum); units in samples; unit values in samples; sample means; squared deviations of sample means from the true mean, $(\bar{y}_t - \mu)^2$; expected value ($E(\bar{y}_{st})$) and variance ($V(\bar{y}_{st})$) of sample mean.

To compare the performances of alternative methods of selecting samples and estimating quantities of interest (alternative **sampling designs**), sampling theorists have devised a number of measures, one of which is **relative efficiency** (RE). For a fixed sample size, n, the relative efficiency of sampling design b compared to sampling design a is defined as:

$$RE(b/a) = V_a(\hat{\Theta})/V_b(\hat{\Theta}) ;$$

where the subscripts denote designs a or b. The relative efficiency of the stratification design in example 2 compared to SRS without stratification (example 1) was about 1.79 (6.50/3.625); the stratification design used in example 3 compared to example 1 resulted in a relative efficiency of only 0.70 (6.50/9.25). Thus, the stratification used in example 2 was almost twice as efficient as SRS (example 1), whereas the stratification used in example 3 was only about two-thirds as efficient as SRS. The stratification used in example 2 was more efficient than SRS in the sense that, for the same sample size and the same amount of sample information, an estimator of nearly twice the precision was obtained.

Multistage Sampling Designs

In the simple examples provided above, it was implicitly assumed that after a pool (unit) was selected, the number of fish in that pool could be counted without error. This assumption normally cannot be met when sampling small streams. Instead, within each selected unit some population estimator must be used to estimate the number of fish present. Thus, estimation of the total number of fish in a small stream requires (at least) two-stage sampling. Units are selected in the first stage, and fish numbers within selected units are estimated in the second stage. **Two-stage sampling designs** are the simplest kinds of **multistage** sampling designs. Estimation of the total number of fish in a moderate-sized or large stream might require three stages of sampling: first stage—selection of several long (10,000 m) sections of stream; second stage—selection of several short (100 m) sections within each long section selected at the first stage; and third stage—use of mark-recapture or removal method population estimators within each short section selected at the second stage. Errors of estimation arise at each stage of sampling, and the mathematical complexity of estimators increases with the number of stages.

This report contrasts the performances of four alternative two-stage sampling designs that could be used to estimate the total number of fish in small streams. Although three stages of sampling may be required for moderate-sized streams, restriction to just two stages of sampling will minimize mathematical complexity and will allow for a sound conceptual understanding of multistage sampling. Two stages of sampling may be entirely adequate for most small streams, or for substantial reaches of larger streams when interest lies solely within those reaches.

Traditional Two-Stage Sampling Design

The traditional approach to estimating the total number of fish in small streams illustrates the simplest type of two-stage sampling design. The total length of a stream is first divided into N sections of equal length, and a simple random sample of n sections is selected. Then, within each selected section (**primary unit**), some population estimator is used to estimate the number of fish present (the **primary unit total**) and to determine an estimated variance for this estimated total. Errors of estimation in this design arise from two sources: (1) extrapolation from the few primary units that are sampled to the entire stream length; and (2) errors of estimation of primary unit totals. The first source

of error is measured by the average variation among (estimated) primary unit totals and is termed **first-stage variance;** the second source of error is measured through (estimated) variances of estimated primary unit totals (based on population estimator formulas) and is termed **second-stage variance.**

Formulas appropriate when using this traditional design, termed a two-stage design with **equal-sized primary units,** are (Bohlin 1981; Cochran 1977, p. 300-303):

$$\hat{Y} = \frac{N}{n} \sum^{n} \hat{Y}_i \, ; \tag{1}$$

$$V(\hat{Y}) = \frac{N\,(N-n)}{n} \, \frac{\sum^{N} (Y_i - \bar{Y})^2}{(N-1)} + \frac{N}{n} \sum^{N} V(\hat{Y}_i) \text{ ; and} \tag{2}$$

$$\hat{V}(\hat{Y}) = \frac{N\,(N-n)}{n} \, \frac{\sum^{n} (Y_i - \hat{\bar{Y}})^2}{(n-1)} + \frac{N}{n} \sum^{n} \hat{V}(\hat{Y}_i) \, ; \tag{3}$$

where: $\bar{Y} = \sum^{N} Y_i/N$, and $\hat{\bar{Y}} = \sum^{n} \hat{Y}_i/n$.

The first term in equation (2) measures first-stage error (variance); the second term measures second-stage error (variance). Equation (2) gives the true variance of the sampling distribution for $\hat{Y}$ and has a single, unique value. In contrast, equation (3) is an estimator for $V(\hat{Y})$ that can take on many possible sample-based values depending on the particular samples that are selected.

A simple numerical example will help illustrate the nature of sample-based calculations for this traditional design. Suppose that a small tributary stream is sampled and that it has a total length of 10,000 m. Five 100-m sections are selected by SRS; within each selected section the two-pass Seber-Le Cren removal method estimator (based on electrofishing; see Everhart and Youngs 1981, p. 107; appendix 3) is used to estimate the number of fish present. Thus, N = 10,000/100 = 100, and n = 5. Suppose that the following estimates are obtained for the sample sections:

Section number		Population estimate ($\hat{Y}_i$)	Estimated variance ($\hat{V}(\hat{Y}_i)$)
1		150	420
2		350	980
3		550	1,540
4		200	560
5		250	700
	Totals	1,500	4,200

Estimated variances are consistent with a fairly low electrofishing capture probability of 0.50. The estimated total number of fish in the stream would be:

$$\hat{Y} = \frac{N}{n} \sum^{n} \hat{Y}_i = \frac{100}{5} \, 1{,}500 = 30{,}000 \, .$$

The estimated variance of this estimated total would be calculated using equation (3):

$$\hat{V}(\hat{Y}) = \frac{N\,(N\text{-}n)}{n} \frac{\sum^{n} (\hat{Y}_i - \hat{\bar{Y}})^2}{(n\text{-}1)} + \frac{N}{n} \sum^{n} \hat{V}(\hat{Y}_i)$$

$$= \frac{100\,(100\text{-}5)}{5} \frac{\sum^{5} (\hat{Y}_i - 300)^2}{(5\text{-}1)} + \frac{100}{5}\,4200$$

$$= 4.75 \times 10^7 + 0.0084 \times 10^7 = 4.758 \times 10^7 .$$

Assuming normality of the sampling distribution for $\hat{Y}$, the 95-percent confidence interval for the total number of fish in the stream would be constructed as:

$$\hat{Y} \pm t_{(n-1),0.95} \sqrt{\hat{V}(\hat{Y})} .$$

This would give 30,000 $\pm$ 2.78·6,898, or 30,000 $\pm$19,176. This is hardly a satisfactory confidence interval for most purposes, but it is based on having sampled only 5 percent of the total stream length.

The important thing here is that virtually all the estimated variance arises from variation among estimated primary unit totals (the first term in equation (3)). The contribution from the second term is negligible, even though electrofishing capture probability is poor and equals 0.50. Had fish in each primary unit been enumerated rather than estimated, there would have been no variance contribution from the second stage of sampling. Estimated variance of the estimated total would have been little affected in this case. The contribution from the first term in equation (3) would remain exactly as it is (assuming that estimated primary unit totals were equal to the true primary unit totals).

This simple example illustrates that errors of extrapolation are likely to be far greater than errors of estimation within selected stream sections. This fact has been generally unappreciated by fishery biologists who have been preoccupied with electrofishing capture probability, or with violations of mark-recapture assumptions (second-stage considerations). The importance of the sampling design itself, as it influences the magnitude of first-stage variance, has been largely ignored.

The traditional two-stage design with equal-sized primary units has at least the following biological and statistical flaws:

1. Selected stream sections, when of equal length, will inevitably include mixtures of habitat types.
2. Placement of block nets to delimit stream sections may displace fish from the section to be sampled. One may often be estimating the number of fish remaining in the section, rather than the original number present.
3. One or both "ends" of selected sections may fall midway in a deep pool where it may be impossible to set block nets. If stream sections were expanded in length, or moved upstream or downstream, in response to this dilemma, a **purposive** decision would

have been made. This would destroy both the intent and the statistical validity of the sampling design itself.

4. The traditional two-stage sampling design generates large first-stage variance and offers only one way to reduce variance of an estimated total. The number of sampled sections must be increased to reduce estimator variance, which will significantly increase the total cost of obtaining estimates.

Alternative Two-Stage Sampling Designs

The large variation among primary unit totals in the traditional two-stage design results because sections, while of equal length, are usually not of equal habitat quality. One section may include primarily riffle habitat, whereas another section may include primarily pool habitat. The total number of fish per primary unit is thus a highly variable and unstable quantity across units because densities of fish per unit area (or per unit volume) vary considerably among habitat types. This makes the squared differences between Y_i and $\bar{Y}$ large (the first term in equation (2)).

Stratification can be effectively used to help reduce first-stage variance. If a stream were mapped into habitat units (entire pools, entire riffles) and units stratified by habitat type, then samples could be drawn independently from each habitat type stratum. Variation among the mean number of fish per unit area (or per unit volume) should be much smaller within each habitat stratum than variation in mean densities of fish between habitat strata. Given estimates of the total number of fish within each stratum ($\hat{Y}_h$), an estimate for the total number of fish in the entire stream can be calculated simply by summing stratum-specific estimates across all strata:

$$\hat{Y} = \sum_{h=1}^{L} \hat{Y}_h ; h = 1, 2, \ldots, L .$$

An estimate for the variance of $\hat{Y}$ can be calculated by summing stratum-specific variance estimates across all strata:

$$\hat{V}(\hat{Y}) = \sum_{h=1}^{L} \hat{V}(\hat{Y}_h) .$$

Simple addition of stratum-specific estimates is justified by the independence of sampling in strata.

If stratification is used, however, then habitat units within each habitat stratum will not be contiguous (for example, a riffle or run would separate two pools within the pool stratum). Also, the habitat units themselves, which are intuitively appealing primary units, will be of variable sizes. If the sizes of natural habitat units are allowed to dictate the sizes of the primary sampling units, then primary units will be of unequal sizes (in contrast to the traditional two-stage design) and the complexity of appropriate sampling designs will be increased. But, allowing primary units to vary in size according to the sizes of the natural habitat units has at least the following advantages:

1. Habitat types will not be mixed among or within sampled primary units because of stratification and because the natural habitat units are equivalent to the primary sampling units.
2. Placement of block nets to delimit primary units will be less likely to displace fish from primary units because fish will tend to seek shelter within their natural habitat units.

3. Estimated numbers of fish in sampled habitat units can be related to the sizes and types of habitat units.
4. Alternative two-stage sampling designs, based on primary units of unequal sizes, can be used to dramatically increase accuracy of estimation of the total number of fish.

The remainder of this report is devoted to a consideration of the costs and benefits of three alternative two-stage sampling designs that are appropriate when primary units are of unequal sizes. It will be assumed that a stream has been mapped and that habitat units have been grouped into two or more habitat type strata. The three alternative designs can be independently applied within strata, and estimated stratum totals and variances can be added across strata to generate estimates for the entire stream. Therefore, it is only necessary to consider application of these alternative designs within a particular stratum; for illustrative purposes, applications are within the pool stratum. Alternative designs could be applied with similar results in other habitat strata.

The three alternative two-stage designs that will be considered may be classified by their selection method and by their use (or lack of use) of an **auxiliary variable.** An auxiliary variable is an attribute of a primary unit that can be inexpensively and easily measured; it can be used to improve precision of estimation of the particular attribute of interest (the target attribute). For small streams, a target attribute is the total number of fish in the pool stratum, and an auxiliary variable is pool size (area or volume). When the numbers of fish present in pools are positively correlated with pool sizes, use of pool size as an auxiliary variable can dramatically improve precision of estimators.

Two of the alternative designs rely on selection of primary units (pools) by simple random sampling. For one of these designs (denoted two-stage SRS) no auxiliary variable is used; for the other design (ratio estimation) the auxiliary variable pool size (area) is used to improve precision of estimators. For the third design, the auxiliary variable pool size is used to calculate the probability of selecting pools. This third method is called selection of primary units with probabilities proportional to their size (PPS). The relative performances of these three alternative designs will first be illustrated using simple, single-stage examples because most of the errors of estimation come from the first stage of sampling (second-stage error is small). That is, fish numbers within pools will initially be enumerated rather than estimated so that there will be no second-stage error. Later, the relative performances of the three alternative designs will be contrasted in a more realistic, two-stage setting.

Alternative sampling designs will be applied to the following small sampling universe of four pools:

Pool number (i)	Pool size (M_i)	Number of fish (Y_i)
1	2	4
2	3	36
3	5	44
4	10	116
Totals	20	200

In each case, samples of size $n = 2$ pools will be drawn from this sampling universe of size $N = 4$ pools. The objective will be to estimate the total number of fish ($Y = 200$) in all four pools. Relevant collective attributes of this sampling universe include: $M_0 = \Sigma M_i = 20$ (total size of all pools); and $R = Y/M_0 = 200/20 = 10$ (average number of fish per unit of pool size). "Size" could be area (m^2) or volume (m^3).

Design A: Two-Stage SRS

According to design A, a sample is drawn from the sampling universe by SRS and the total number of fish in all pools is estimated as:

$$\hat{Y}_{srs} = \frac{N}{n} \sum^{n} Y_i \, .$$

The six possible SRS samples, estimated totals for each sample ($\hat{Y}_t$), and squared deviations between estimated totals and the true total $(\hat{Y}_t - Y)^2$ are as follows:

Sample units		$\hat{Y}_t$	$(\hat{Y}_t - Y)^2$
1,2		80	14,400
1,3		96	10,816
1,4		240	1,600
2,3		160	1,600
2,4		304	10,816
3,4		320	14,400
	Totals	1,200	53,632

Each of these samples is equally likely (because selection is by SRS), so the expected value of $\hat{Y}_{srs}$ may be calculated as:

$$E(\hat{Y}_{srs}) = \sum_{t=1}^{6} \hat{Y}_t P_t = (1/6) \cdot 1{,}200 = 200 = Y \, .$$

This design results in an unbiased estimator ($E(\hat{Y}_{srs}) = Y$), so variance of the estimated total (here equivalent to mean square error) can be calculated as:

$$V(\hat{Y}_{srs}) = \sum_{t=1}^{6} (\hat{Y}_t - Y)^2 P_t = (1/6) \cdot 53{,}632 = 8{,}939 \, .$$

Design B: Ratio Estimation

For design B, primary units are selected by SRS, but a measure of the size of selected units (an auxiliary variable) is incorporated into estimators. The total number of fish in all pools is now estimated as:

$$\hat{Y}_{rat} = M_0 \sum^{n} Y_i / \sum^{n} M_i = M_0 \hat{R} \, ;$$

where $\hat{R} = \sum^{n} Y_i / \sum^{n} M_i$.

Ratio estimation has a simple intuitive basis. Based on the sample, one obtains an estimate of the true number of fish per unit of pool size (R). An estimator for the total number of fish in all pools would be the total size of all pools (M_0) times the estimated number of fish per unit of pool size ($\hat{R}$, usually called the sample ratio). The six possible SRS samples, estimated sample ratios ($\hat{R}_t$) and totals ($\hat{Y}_t$), and squared deviations between estimated totals and the true total ($(\hat{Y}_t - Y)^2$) are:

Sample units	$\hat{R}_t$	$\hat{Y}_t$	$(\hat{Y}_t - Y)^2$
1,2	8	160	1,600
1,3	6.86	137	3,951
1,4	10	200	0
2,3	10	200	0
2,4	11.69	234	1,145
3,4	10.67	213	178
Totals		1,144	6,874

The expected value of the ratio estimator for the total number of fish in all four pools would be:

$$E(\hat{Y}_{rat}) = \sum_{t=1}^{6} \hat{Y}_t P_t = (1/6)\cdot 1,144 = 190.7 \neq 200 .$$

Thus, the ratio estimator is biased:

$$BIAS(\hat{Y}_{rat}) = E(\hat{Y}_{rat}) - Y = 190.7 - 200 = -9.3 .$$

For that reason, the appropriate measure of accuracy is mean square error rather than variance:

$$MSE(\hat{Y}_{rat}) = \sum_{t=1}^{6} (\hat{Y}_t - Y)^2 P_t = (1/6)\cdot 6,874 = 1,146 .$$

Variance of the ratio estimator could be calculated as:

$$V(\hat{Y}_{rat}) = \Sigma[\hat{Y}_t - E(\hat{Y}_{rat})]^2 P_t;$$

but it is more easily calculated as the difference between mean square error and squared bias:

$$V(\hat{Y}_{rat}) = MSE(\hat{Y}_{rat}) - [BIAS(\hat{Y}_{rat})]^2 = 1,146 - (-9.3)^2 = 1059.5 .$$

Although the ratio estimator is slightly biased, variance is sufficiently small so that the accuracy of design B is considerably greater than for design A. This improvement comes from use of the auxiliary variable pool size (M_i), and from the positive correlation between fish numbers and pool sizes. Estimated totals ($\hat{Y}_{rat}$) ranged from only 137 to 234 among all samples (as compared to a range of from 80 - 320 for design A), and variance was dramatically reduced as a result. This contrast in performance between designs A and B provides a clear example of when a biased estimator might be preferred over an unbiased estimator.

Design C: PPS Without Replacement

For design C, primary units are selected by PPS without replacement. Although the same possible samples result, by this method of selection all possible samples are not equally likely (as they are using SRS selection). Larger pools are more likely to be included in samples than are smaller pools. There are many possible ways to select samples by PPS without replacement, but all selection methods produce two kinds of probability assignments:

π_i = probability that unit i is in a sample of size n; and
π_{ij} = probability that units i and j are in a sample of size n.

When PPS without replacement is used to select samples, the total number of fish in all pools is estimated as:

$$\hat{Y}_{pps} = \sum^{n} Y_i/\pi_i \ .$$

For illustrative purposes, PPS selection probabilities will be based on a method in which successive units are selected with probabilities proportional to the sizes of the remaining units (appendix 1 contains a summary of computations for this method). **First-order inclusion probabilities** (the π_i's) for the pool sampling universe, for samples of size n = 2 pools, are:

Unit number	Size (M_i)	π_i
1	2	0.2510
2	3	.3666
3	5	.5718
4	10	.8104

The largest pool is more than three times as likely to be in the sample (π_4 = 0.8104) than the smallest pool (π_1 = 0.2510).

When n = 2, then the **second-order inclusion probabilities** (the π_{ij}'s) are the same as the probabilities of individual samples. The six possible PPS without replacement samples, estimated total ($\hat{Y}_t$), squared deviations between estimated totals and the true total ($(\hat{Y}_t - Y)^2$), and sample probabilities ($P_t = \pi_{ij}$) for the pool sampling universe are:

Sample units	$\hat{Y}_t$	$(\hat{Y}_t - Y)^2$	$P_t = \pi_{ij}$
1,2	114.20	7,362	0.0343
1,3	92.89	11,473	.0611
1,4	159.08	1,675	.1556
2,3	175.15	617	.0941
2,4	241.34	1,709	.2382
3,4	220.09	403	.4167

Note that $\Sigma P_t = 1$ (as in SRS) and that sample probabilities vary and reflect sizes of pools. The expected value of the PPS without replacement estimator can be calculated as:

$$E(\hat{Y}_{pps}) = \sum_{t=1}^{6} \hat{Y}_t P_t = 114.20 \cdot 0.0343 + 92.89 \cdot 0.0611 + \ldots + 220.09 \cdot 0.4167 = 200.0 = Y\,.$$

Because $\hat{Y}_{pps}$ is unbiased, variance of the estimator can be calculated as:

$$V(\hat{Y}_{pps}) = \sum_{t=1}^{6} (\hat{Y}_t - Y)^2 P_t$$

$$= 7{,}362 \cdot 0.0343 + 11{,}473 \cdot 0.0611 + \ldots + 403 \cdot 0.4167 = 1{,}847.$$

Variance for the PPS without replacement estimator [$V(\hat{Y}_{pps}) = 1{,}847$] is substantially less than for the two-stage SRS estimator [$V(\hat{Y}_{srs}) = 8{,}939$] and the PPS estimator is also unbiased. But because accuracy of the PPS estimator is slightly less than for the ratio estimator [$MSE(\hat{Y}_{rat}) = 1{,}146$], it would be difficult to choose between designs B and C on the basis of accuracy alone.

Determining the Best Choice Among Alternative Designs

The preceding applications of the three alternative designs allowed a comparison of the accuracies and degrees of bias of the three estimators. Choice among the alternative designs must also include, however, a consideration of the total costs of obtaining estimates, usually termed **total survey costs.** Just as one can compare the relative efficiencies of alternative designs, one can also compare the **relative cost** (RC) of one design to another. The relative cost of design b compared to design a is defined (for a fixed sample size, n) as:

$$RC(b/a) = C_b/C_a\,;$$

where C_a and C_b are the total survey costs for designs a and b.

Total survey costs for estimating the total number of fish in small streams can be separated into two distinct categories: (1) costs that are independent of the particular selected units; and (2) costs that directly depend on the particular selected units. The first category (fixed costs) includes housing, per diem and travel (to and from the study site, and between selected units), and time spent setting up and taking down block nets if electrofishing is used in selected units. The second category includes time actually spent in selected units to estimate fish numbers.

The average total size of n units selected by PPS without replacement will be greater than the average total size of n units selected by SRS because the PPS design assigns higher selection probabilities to larger units. Because it takes longer to sample a large pool than a small pool, it will cost more to sample the same number of pools when they are selected by PPS (design C) than when they are selected by SRS (designs A and B).

For the traditional, equal-sized primary unit design, total costs of a stream survey are probably roughly split in half between the two cost categories. It seems reasonable to assume that this would also be the case if unequal-sized primary units were selected by SRS. This assumption leads to a simple cost function of the form:

$$C_{srs} = 0.5\ C_{srs} + \alpha\ X_{srs}\ ;$$

where: X_{srs} = expected (average) total size of n units selected by SRS; and
α = cost per unit of size, such that $\alpha\ X_{srs} = 0.5\ C_{srs}$.

A comparable cost function for the PPS design would be:

$$C_{pps} = 0.5\ C_{srs} + \alpha\ X_{pps}\ ;$$

where X_{pps} = expected (average) total size of n units selected by PPS without replacement.

The expected (average) total size of n units selected by SRS is:

$$X_{srs} = n \sum^{N} M_i/N\ ;$$

whereas that for n units selected by PPS without replacement is:

$$X_{pps} = \sum^{N} M_i \pi_i\ .$$

Expected total sizes of selected units are used for comparisons because they reflect the overall average behavior of the design. Actual total sizes of selected units would depend on the particular samples selected.

The RC of the PPS design (design C) compared to the SRS designs (A and B) can be determined by normalizing the total cost of the SRS designs. That is, let $C_{srs} = 1$. Then, $\alpha = 0.5/X_{srs}$, and the expected total cost of the PPS design would be $C_{pps} = 0.5 + \alpha X_{pps}$. RC(PPS/SRS) would then equal C_{pps}/C_{srs}. This procedure can be illustrated using the same small sampling universe of four pools. The expected total size of two of these four units selected by SRS was $X_{srs} = n \sum M_i/N = 2 \cdot 20/4 = 10$; so that $\alpha = 0.5/10 = 0.05$. The expected total size of two units selected by PPS without replacement was:

$$X_{pps} = \sum^{4} M_i \pi_i = 2 \cdot 0.2510 + 3 \cdot 0.3666 + 5 \cdot 0.5718 + 10 \cdot 0.8104 = 12.5648\ .$$

The expected total cost of the PPS design was:

$$C_{pps} = 0.5 + 0.05 \cdot 12.5648 = 1.128;$$

and RC(PPS/SRS) = $C_{pps}/C_{srs} = 1.128/1 = 1.128 = C_{pps}$. Thus, normalizing total survey costs made C_{pps} = RC(PPS/SRS).

Finally, **net relative efficiency** (NRE) is a measure of the cost-effectiveness of alternative designs and is defined (for a fixed sample size, n) as:

$$NRE(b/a) = RE(b/a)/RC(b/a) = V_a(\hat{\Theta})C_a/V_b(\hat{\Theta})C_b .$$

For the small sampling universe used for examples, the net relative efficiency of ratio estimation as compared to the two-stage SRS design is equivalent to the relative efficiency because total survey costs are the same. The net relative efficiency of the PPS design compared to the SRS design, however, would be NRE(PPS/SRS) = 8939·1/1847·1.128 = 4.29; and the net relative efficiency of the PPS design compared to the ratio estimation design would be NRE(PPS/ratio) = 1059·1/1847·1.128 = 0.508. Thus, in this case, the PPS design was about four times as cost-effective as the two-stage SRS design, but only about half as cost-effective as the ratio estimation design. Choice of sampling design should be based on net relative efficiency because it balances possible improvements in efficiency (that is, reductions in variance or mean square error) against possible increases in total survey costs. On the basis of net relative efficiency, the ratio estimation design would be the design of choice for the small sampling universe of four pools (but see "Discussion").

Realistic Applications

The three alternative two-stage sampling designs were applied in their full two-stage forms to a realistic, large sampling universe (N = 50) constructed on the basis of data collected from Knowles Creek, a small-stream tributary to the Siuslaw River in Oregon. A two-pass electrofishing/removal method estimator was assumed to have been used to estimate the number of fish in selected primary units and electrofishing capture probability was set to 0.50. Relevant formulas for the full two-stage designs are presented in appendix 2, and details of their application are presented in appendix 3.

Figure 2 shows a plot of the number of fish (yearling coho salmon, *Oncorhynchus kisutch*) in Knowles Creek pools against the sizes (areas, in m^2) of pools. Although there is a substantial amount of variation in fish numbers for a given pool size, there is a significant increasing trend of fish numbers with pool size. Pool size accounts for roughly 50 percent of the variation in fish numbers (R^2 = 0.58 for a linear regression of fish number against pool size).

Figure 3 shows sampling variances (or mean square error) for two-stage SRS, ratio estimation, and PPS without replacement sampling designs as a function of sample size. Sampling variances of alternative designs follow a consistent pattern over all sample sizes: variance of the SRS design is greatest; that of ratio estimation is noticeably less and is, essentially, a constant fraction of variance for the SRS design; and variance of the PPS design is substantially less than for ratio estimation. The magnitude of variance for the PPS design, compared to the other designs, generally improves with sample size.

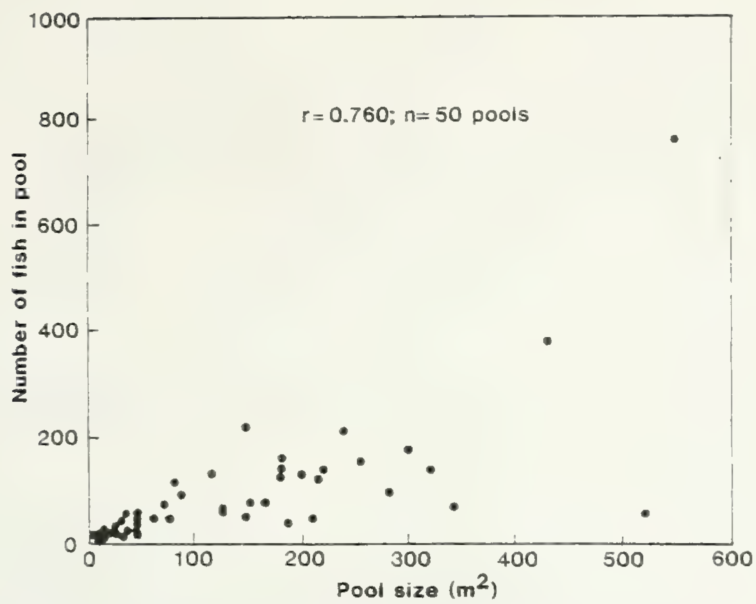

Figure 2.—Total number of fish plotted against pool size for the sampling universe used for realistic applications of alternative two-stage sampling designs. Based on data collected from Knowles Creek, Oregon, by the USDA Forest Service.

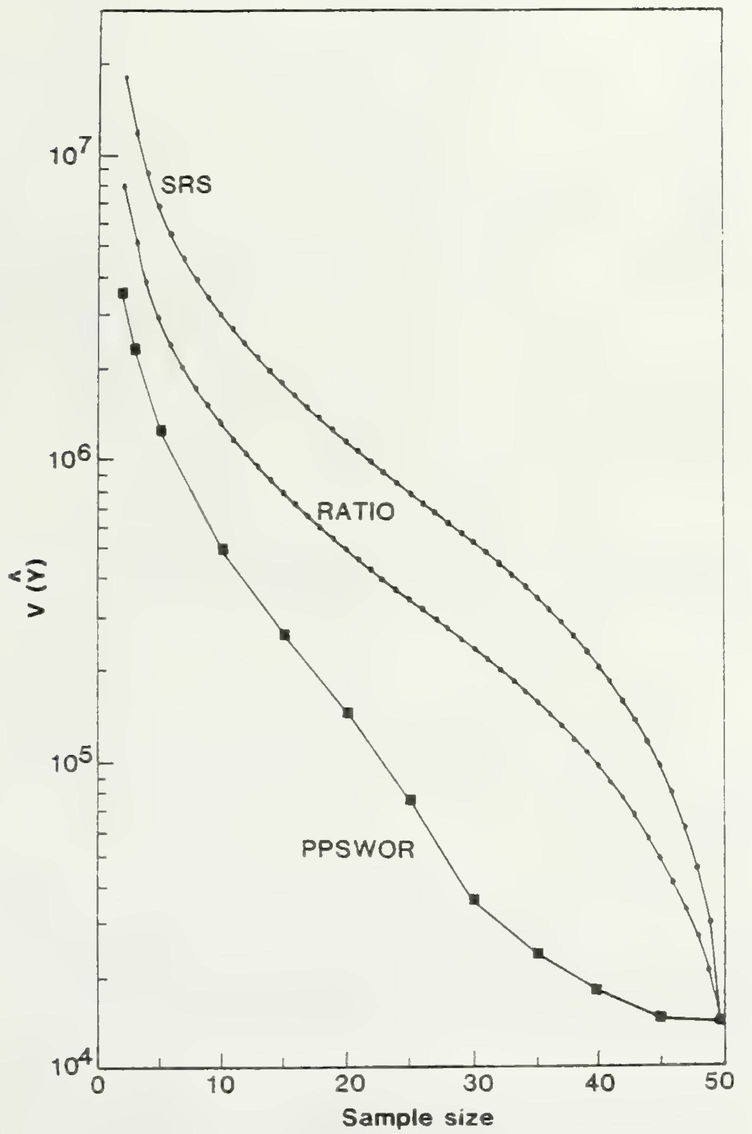

Figure 3.—Sampling variances ($Y(\hat{Y})$) for two-stage SRS (SRS), ratio estimation (RATIO) and PPS without replacement (PPSWOR) sampling designs plotted against sample size for the Knowles Creek sampling universe. Values plotted for the ratio estimation design are actually MSE($\hat{Y}$) because the design is biased.

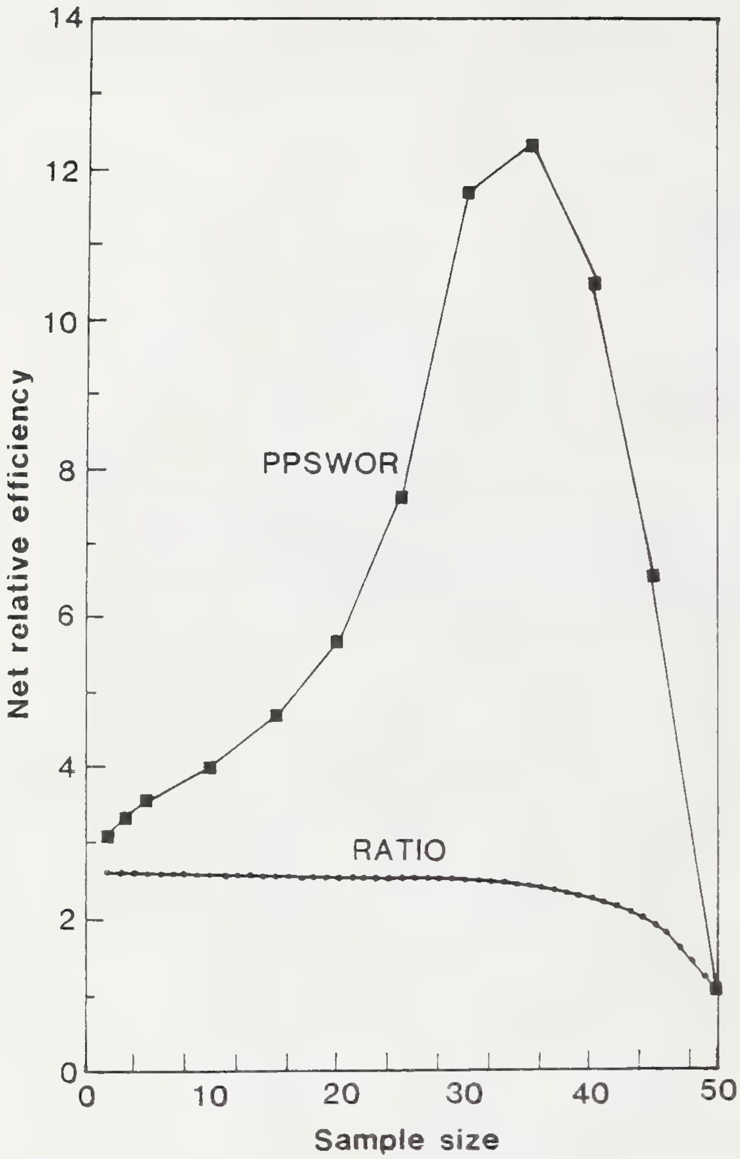

Figure 4.—Net relative efficiencies of PPS without replacement (PPSWOR) and ratio estimation (RATIO) designs (as compared to the two-stage SRS design) plotted against sample size for the Knowles Creek sampling universe.

Figure 4 shows the net relative efficiencies of the ratio estimation and PPS designs compared to the SRS design as a function of sample size. Net relative efficiency of ratio estimation is about 2.7 and is essentially independent of sample size (for $n < 40$). For the PPS design, net relative efficiency increases with sample size until it exceeds 12 when $n = 35$. Based on the criterion of cost-effectiveness, both ratio estimation and PPS without replacement offer substantial improvements over the SRS design. Depending on sample size, the PPS without replacement design could be more than 10 times as cost-effective as the SRS design.

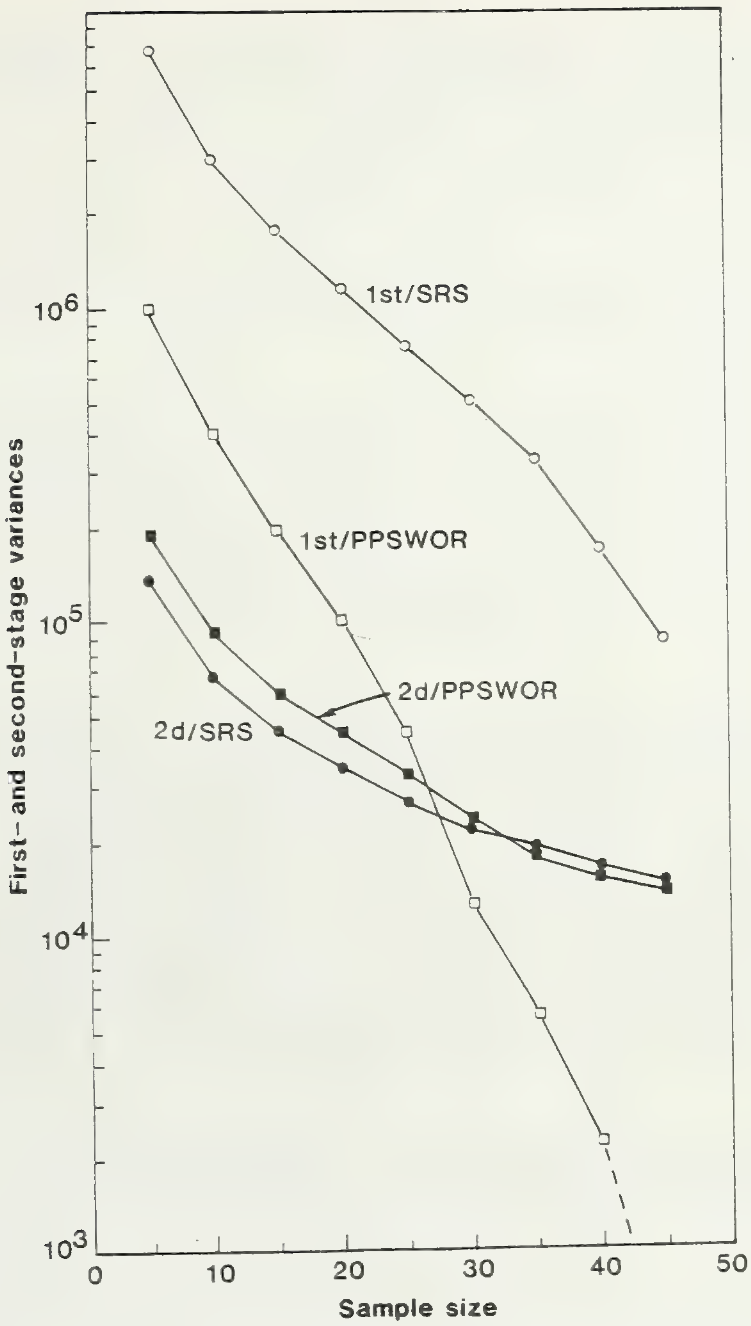

Figure 5.—First- and second-stage sampling variances for the two-stage SRS (SRS) and PPS without replacement (PPSWOR) designs plotted against sample size for the Knowles Creek sampling universe.

Figure 5 shows that the striking performance of the PPS design was achieved entirely through dramatic and rapid reduction of first-stage variance with increasing sample size. First-stage variance was almost always at least an order of magnitude larger than second-stage variance for the SRS design. In contrast, first-stage variance rapidly decreased for the PPS design until (for $n \geq 25$) it was actually less than second-stage variance. The PPS design effectively addressed the usually large first-stage variance problem that is associated with the traditional two-stage design having equal-sized primary units.

Discussion

The simple single-stage examples and the more realistic two-stage examples presented here illustrate the importance of sampling design in determining errors of estimation of the total number of fish in small streams. Although fishery biologists have paid a great deal of attention to errors of estimation within selected sample sections (second-stage variance), they have not devoted much attention to errors of extrapolation from the small number of sections sampled to an entire stream (first-stage variance). Most fishery biologists receive extensive training in population estimation and this training can be used to help reduce second-stage variance. Reduction of first-stage variance requires knowledge of sampling theory, however, and few fishery biologists receive formal training in this area.

The traditional two-stage sampling design with equal-sized primary units (equal-length sections of stream constitute the primary sampling units) results in large first-stage variance. Although primary units are of equal size, they may vary greatly in habitat quality and in the number of fish that they can support. The only way to reduce this large first-stage variance when using the traditional design is to increase the number of sampled sections. This significantly increases total survey costs.

Stratification of stream habitat into pools, riffles, and other habitat types, coupled with independent sampling in each constructed stratum, can help reduce first-stage variance by limiting variation in habitat quality among sampled sections. When stratification is employed, however, the primary units become equivalent to the natural habitat units and are then of unequal sizes. The unequal sizes of primary units increase the complexity of applicable sampling designs, but also offer a wide choice of alternative designs. Of three such alternative designs considered here, both ratio estimation and PPS without replacement appear to have considerable promise for improving the precision and accuracy of estimates of the total number of fish in small streams. Both designs take advantage of the usually strong positive correlation between fish numbers and habitat unit sizes; greater numbers of fish are usually found in large pools than in small pools.

Evaluation of the relative performances of alternative designs required their application to specific sampling universes and calculation of the expected (average) behavior of estimators. Because total survey costs varied among designs (as a result of primary unit selection method), it was necessary to calculate total survey costs in addition to estimator sampling variance (or mean square error) in order to calculate a measure of the cost-effectiveness of alternative designs. Choice of design should be based on cost-effectiveness (net relative efficiency) rather than on accuracy alone.

The simple cost functions used in this report could be made more realistic in two respects. First, time spent electrofishing a selected unit may not be linearly related to pool (or riffle) area, but may instead increase as some power of pool area. This possibility was examined by Hankin (1984) but had little impact on relative performances of alternative designs. Second, it may be necessary to add an additional term to cost functions to account for the different stream mapping requirements of the alternative designs. When primary units are of unequal sizes, the three alternative sampling designs (as presented in this report) all require a map of the locations of all primary units. This is necessary so that the sampling universe can be specified. Without a specified sampling universe, it is impossible to compare the expected performances of alternative designs. The quality of maps required for the different designs may vary substantially, however. For the two-stage SRS design, only the location of the primary units is required and there is no need to measure primary unit sizes. In contrast, both ratio estimation

and PPS without replacement designs require some measure of primary unit sizes in addition to locations. Increased mapping expenses for the ratio estimation and PPS designs would increase the relative costs for these designs compared to the SRS design. The calculations of cost-effectiveness presented in this report should therefore be viewed with some skepticism.

Ratio estimation requires (1) the sizes of primary units that appear in the sample of n units, and (2) the total size of all primary units. In a practical sense, the latter requirement means that the sizes of all primary units have to be determined. The PPS design requires either accurate measurements of all primary unit sizes, or some less accurate measurements of primary unit sizes that are highly correlated with the true primary unit sizes. For example, if a reach of stream were of fairly uniform width, then length of pool would be highly correlated with pool area. Alternatively, visual ("eye") estimates of primary unit sizes could be used to assign PPS selection probabilities if estimates were highly correlated with the true sizes. The marginal cost of obtaining these simpler measurements of primary unit sizes would probably be small compared to costs of locating primary units (which must be done for all designs).

There is an additional way to reduce mapping costs for both SRS and ratio estimation designs that was not considered in this report but that has substantial merit. Instead of selecting primary units by SRS, one could instead select units by **systematic** sampling. In systematic sampling, one chooses a random start from the integers 1 through K, and then selects every K^{th} unit. In the context of a stream survey, 1/K could be the desired sampling intensity (fraction of habitat units that are sampled). No map is needed for systematic sampling. A field crew could proceed upstream or downstream and pick, say, every 10th pool (or riffle) for sampling. When the survey was complete, the total number of pools (or riffles) would be known and, if each sampled unit were measured, the total size of all habitat units could be estimated.

The systematic sampling approach would allow valid application of the two-stage SRS design and (with some minor modifications) the ratio estimation design. The SRS design would still be unbiased, but systematic sampling would rule out unbiased estimation of variance. For that reason, the systematic sampling approach was not formally presented in this report. Nevertheless, systematic sampling could prove practical and cost-effective. It will rarely perform worse than SRS, and it will usually perform better. Formulas presented for the two-stage SRS design could also be used if units were selected by systematic sampling; they would tend to overestimate the true sampling variance and would therefore provide conservative estimates of sampling variance.

In many situations, fishery biologists may not have any preliminary data with which to construct plausible sampling universes and thereby judge the probable performances of alternative designs prior to field work. In such instances, it seems most reasonable to first sample using the SRS design and then to compare estimated variances of the SRS design (equation 3, appendix 2) with estimated mean square error of the ratio estimation design (equation 6, appendix 2). Because the sample-based estimator of mean square error for ratio estimation requires only measurements of those units that are selected,the cost of making this comparison would be small. If sample sizes exceeded about 12 (see appendix 2; table 1), then the comparison would be valid. If it showed that mean square error for ratio estimation was far less than for the two-stage SRS design, then it would be worth pursuing the ratio estimation or PPS designs (with their increased mapping expenses) in future survey work. Use of the PPS design without preliminary data is

Table 1—Guidelines for use, mapping needs, probable relative survey costs (compared to the SRS design), and restrictions and/or requirements for alternative two-stage sampling designs with unequal-sized primary units

Design	Conditions when design should be effective	Mapping requirements	Total survey costs (for fixed sample size)	Restrictions and/or requirements
Two-stage SRS	Weak correlation between fish numbers and primary unit sizes ($r < 0.4$)	Location of all primary units	Base for comparison of alternative designs	Can be used for any size sampling universe and any sample size
	Small range (< 4-fold) in primary unit sizes	Stratification by primary unit sizes may improve performance		Always use for preliminary surveys
Ratio estimation	Strong correlation between primary unit sizes and fish numbers ($r > 0.5$)	Location of all primary units	Greater: increased mapping costs	Can be used for any size sampling universe
	Substantial range (> 4-fold) in primary unit sizes	Sizes of all primary units in sample		Sample size should exceed 12
		Total size of all units		
PPS without replacement	Moderate correlation between fish numbers and primary unit sizes ($r > 0.4$)	Location of all primary units	Greater: increased mapping costs	Sampling universe should be no larger than $N = 50$ to 100
	Substantial range (> 4-fold) in primary unit sizes	Suitable measure of sizes of all primary units; may be visual estimates	Average size of units in sample will be larger than for SRS	Computer required for all computations
				Biometrician should be consulted prior to use

definitely not recommended. Table 1 summarizes those conditions under which alternative designs should be effective, their probable costs, and their requirements and/or restrictions.

Regardless of the choices made among alternative sampling designs, the practice of allowing natural habitat units to dictate the primary sampling units seems wise. Displacement of fish from sampled sections due to setting block nets, mixture of habitat types within sampled sections, and the impossibility of placing block nets in deep pools should all be minimized or eliminated. Analysis of estimates generated from distinctive habitat unit types of varying sizes can allow one to draw important conclusions regarding

relationships among habitat unit sizes and types and fish abundance. These conclusions are difficult, if not impossible, to draw when primary units are of equal sizes. Adoption of unequal-sized primary unit sampling designs should improve accuracy of estimation of fish numbers, but more importantly it should improve our understanding of the dynamics of fish populations in small streams.

English Equivalents

1 meter (m) = 39.37 inches or 3.28 feet
1 square meter (m_2) = 10.7639 square feet

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Appendix 1

Computation of Selection Probabilities for the PPS Design

All PPS selection methods require calculation of (at least) the following inclusion probabilities:

π_i = probability that unit i is the sample ($i = 1, 2, \ldots, N$); and
π_{ij} = probability that units i and j are in the sample ($i \neq j$).

When samples are of size $n = 2$, then π_{ij} is equivalent to P_t, where P_t is the probability of drawing the t^{th} sample (that particular sample that contains the units i and j). Inclusion probabilities will depend on three things: (1) sample size, n; (2) the sizes of units in the sampling universe (or some other measurement of unit size that is used to assign selection probabilities to units); and (3) the particular PPS without replacement selection method that is used. Selection probabilities for the case of sampling $n = 2$ from $N = 4$, used for illustrative purposes in this report, were calculated using a selection method by which units are selected with probabilities proportional to the sizes of the remaining units.

Calculations for the single stage example in design C were as follows:

1. Calculate $p_i = M_i/M_0$. These are the probabilities that unit i will be selected on the first draw: $M_0 = \sum^{N} M_i$.
2. Calculate $p(j|i) = p_j/(1-p_i)$. These are the **conditional** probabilities of drawing the j^{th} unit on the second draw given that unit i was selected on the first draw. These conditional probabilities are equivalent to those probabilities that would be calculated on the basis of the sizes of the remaining units at the time of the second draw. The table below lists the $p(j|i)$ for all possible orders of selection:

Units selected		
First draw (i)	Second draw (j)	Conditional Probabilities: p(j|i)
1	2	0.1666
1	3	.2777
1	4	.5555
2	1	.1176
2	3	.2941
2	4	.5882
3	1	.1333
3	2	.2000
3	4	.6666
4	1	.2000
4	2	.3000
4	3	.5000

3. Calculate $\pi_{ij} = P_t = p_i \cdot p(j|i) + p_j \cdot p(i|j)$. The two terms in this sum give the probabilities of drawing the ordered samples (i, j) and (j, i). That is, for example, the ordered sample (2, 3) would be drawn as:

A. Probability of drawing unit 2 on first draw = $p_2 = 0.15$.
B. Probability of drawing unit 3 on second draw given that unit 2 was drawn on first draw = $p(3|2) = 0.2941$.

Thus, the probability of drawing unit 2 on the first draw and then unit 3 on the second draw would be: $0.15 \cdot 0.2941 = 0.044115$. Similarly, the probability of drawing the ordered sample (3, 2) would be: $p_3 \cdot p(2|3) = 0.25 \cdot 0.20 = 0.0500$.

The probability of the particular sample that contained the units 2 and 3, without regard to order, would then be obtained as the sum of the probabilities of the two possible ordered samples:

$$\pi_{2,3} = \pi_{3,2} = p_2 \cdot p(3|2) + p_3 \cdot p(2|3)$$
$$= 0.044115 + 0.0500 = 0.094115.$$

Analogous computations resulted in the figures presented for π_{ij} in the single-stage example for the PPS without replacement design (page 15).

Selection of primary units with probabilities proportional to the sizes of remaining units is a method that cannot be easily extended to selection of large samples from large sampling universes. In practice, the requirement that probabilities of all possible ordered samples be calculated in order to obtain the probabilities for the unordered samples rules out application of this method for any but small samples ($n \leq 5$) drawn from small sampling universes ($N \leq 25$). The sheer magnitude of necessary calculations quickly exceeds any reasonable expectations for modern computers. For example, the number of ordered samples of size $n = 10$ selected from a universe of size $N = 50$ is $N!/(N-n)! = 3.73 \times 10^{16}$.

For the more realistic two-stage applications of the alternative designs contrasted in this report, selection of PPS samples was made according to a method developed by Chao (1982). This selection method does not require construction of all possible ordered samples and it can be readily extended to selection of large sample sizes from large sampling universe. The method does not perform well, however, for very small sample sizes (usually $n \leq 3$ or 4) because some π_{ij} may be zero.

By using either selection with probabilities proportional to the sizes of the remaining units or Chao's method, as appropriate, one can effectively use PPS without replacement designs for small- and moderate-sized sampling universes ($N \leq 100$). When $N > 100$, current computing time and costs may rule out use of this design. Given the rates of advancement in computer technology, however, methods such as Chao's will probably be extendable to much larger sampling universes in the near future. Additional technical details concerning the above two PPS without replacement selection methods can be found in Hankin (1984, appendix B).

Appendix 2

Two-Stage Estimators for Alternative Designs

When there are two stages of sampling, primary units are of unequal sizes, and some population estimator is used to generate estimated primary unit totals ($\hat{Y}_i$) and estimated variances for the estimated totals ($\hat{V}(\hat{Y}_i)$), the appropriate estimators are as follows:

Design A.—Two-stage SRS (these are the same as those for the traditional design with equal-sized primary units):

$$\hat{Y}_{srs} = \frac{N}{n} \sum^{n} \hat{Y}_i; \tag{1}$$

$$V(\hat{Y}_{srs}) = \frac{N\,(N\text{-}n)}{n} \frac{\sum^{N}(Y_i - \bar{Y})^2}{(N\text{-}1)} + \frac{N}{n} \sum^{N} V(\hat{Y}_i); \text{ and} \tag{2}$$

$$\hat{V}(\hat{Y}_{srs}) = \frac{N\,(N\text{-}n)}{n} \frac{\sum^{n}(Y_i - \hat{\bar{Y}})^2}{(n\text{-}1)} + \frac{N}{n} \sum^{n} \hat{V}(\hat{Y}_i). \tag{3}$$

Design B.—Ratio estimation (see Cochran 1977, p. 300-305):

$$\hat{Y}_{rat} = M_0 \sum^{n}\hat{Y}_i / \sum^{n} M_i; \tag{4}$$

$$MSE(\hat{Y}_{rat}) \approx \frac{N\,(N\text{-}n)}{n} \frac{\sum^{N} M_i^2(\bar{Y}_i - \bar{\bar{Y}})^2}{(N\text{-}1)} + \frac{N}{n} \sum^{N} V(\hat{Y}_i); \text{ and} \tag{5}$$

$$\widehat{MSE}(\hat{Y}_{rat}) \approx \frac{N\,(N\text{-}n)}{n} \frac{\sum^{n} M_i^2(\hat{\bar{Y}}_i - \hat{\bar{\bar{Y}}})^2}{(n\text{-}1)} + \frac{N}{n} \sum^{n} \hat{V}(\hat{Y}_i)\,. \tag{6}$$

where: $\bar{Y}_i = Y_i/M_i$;

$\bar{\bar{Y}} = \sum^{N} Y_i / \sum^{N} M_i = Y/M_0$;

$\hat{\bar{Y}}_i = \hat{Y}_i/M_i$; and

$\hat{\bar{\bar{Y}}} = \sum^{n} Y_i / \sum^{n} M_i$.

Both equations (5) and (6) are large-sample approximations to mean square error of $\hat{Y}_{rat}$ and should be used only for $n > 12$ (Cochran 1977, p. 162-164).

Design C.—PPS without replacement (see Raj 1968, p. 118-119):

$$\hat{Y}_{pps} = \sum^{n} \hat{Y}_i/\pi_i; \tag{7}$$

$$V(\hat{Y}_{pps}) = \sum_{i}^{N-1}\sum_{j>i}^{N} (\pi_i\pi_j-\pi_{ij})(Y_i/\pi_i-Y_j/\pi_j)^2 + \sum^{N} V(\hat{Y}_i)/\pi_i; \text{ and} \tag{8}$$

$$\hat{V}(\hat{Y}_{pps}) = \sum_{i}^{n-1}\sum_{j>i}^{n} \frac{(\pi_i\pi_j-\pi_{ij})}{\pi_{ij}} (\hat{Y}_i/\pi_i - Y_j/\pi_j)^2 + \sum^{n} \hat{V}(\hat{Y}_i)/\pi_i\ ; \tag{9}$$

with the restrictions that:

all $\pi_{ij} > 0$; $\sum^{N} \pi_i = n$; and $\sum_{i}^{N-1}\sum_{j>i}^{N} \pi_{ij} = n(n-1))/2$.

Summations in equations (8) and (9) are over all distinct pairs of primary units in the sampling universe (equation (8)) or in the sample (equation (9)).

Equations (1)-(3) and (7)-(9) are formally unbiased when unbiased estimators are used at the second stage of sampling. Equations (4)-(6) are biased and approximate, as is always the case for ratio estimators. In practice, all formulas will be only approximately correct because there are no existing population estimators (used at the second stage of sampling) that are unbiased. The formulas can still be used with confidence, however, because second-stage error is usually small compared to first-stage error, and BIAS($\hat{Y}_i$) is usually minor for both removal method and mark-recapture population estimation.

The particular population estimator used at the second stage of sampling is unspecified in the above formulas. Any valid population estimator could be used and formulas would be unaffected. The particular population estimator used at the second stage of sampling will only affect the form of the equations used to calculate $V(\hat{Y}_i)$ and $\hat{V}(\hat{Y}_i)$.

Appendix 3

Details of the Realistic Application of Alternative Designs

Application of the alternative two-stage sampling designs in a realistic setting required (1) construction of a realistic sampling universe; (2) adoption of a particular population estimator at the second stage of sampling; and (3) determination of sampling design performance when applied to that sampling universe.

The realistic sampling universe was constructed on the basis of sampling data collected from Knowles Creek, a tributary of the Siuslaw River in Oregon. Based on electrofishing/removal method estimation, fishery biologists of the Pacific Northwest Research Station provided estimated population sizes and pool sizes (areas, in m^2) for pools ranging in size from about 4 m^2 to 600 m^2. These data were used to construct a large sampling universe ($N = 50$) by assuming that estimated numbers in pools were exactly equal to the numbers actually present in sampled pools. The correlation between fish numbers and pool sizes ($r = 0.76$) is probably a quite reasonable figure to expect. In some cases the correlation may be larger, and in other cases it may be less.

A two-pass electrofishing/removal method estimator was assumed used to estimate population size (Y_i) within sampled sections. Letting C_1 = number of fish caught on the first pass; C_2 = number of fish caught on the second pass; and q = probability of capture, then (Seber 1982, sec. 7.2):

$$\hat{Y}_i = C_1/(C_1 - C_2) \; ; \tag{10}$$

$$V(\hat{Y}_i) \approx Y_i(1-q)^2(2-q)q^{-3} \; ; \text{ and} \tag{11}$$

$$\hat{V}(\hat{Y}_i) = C_1C_2(C_1 + C_2)/(C_1 - C_2)^4 \; . \tag{12}$$

Probability of capture was set equal to 0.50, a fairly low figure, so as not to minimize the magnitude of second-stage error.

Actual application of the three alternative designs involved:

1. Calculation of sampling variance (or mean square error) for each design as a function of sample size using formulas (2) and (11), (5) and (11), and (8) and (11).
2. Calculation of relative costs for each design (compared to the two-stage SRS design) as a function of sample size using the cost function presented in this report.
3. Calculation of net relative efficiencies for each design (compared to the two-stage SRS design) as a function of sample size.

All necessary calculations were performed on a large time-sharing computer (CYBER) using programs written by the author in APL. All designs have also been implemented, however, on a less powerful machine (COMPAQ DESKPRO, an IBM-compatible microcomputer). [1/] With the exception of drawing large samples for the PPS without replacement design, the microcomputer would be entirely adequate for all calculations.

[1/] Use of a trade name does not imply endorsement or approval of any product by the USDA Forest Service to the exclusion of others that may be suitable.

Appendix 4

Estimation of Total Biomass

There are many possible alternative methods for estimating the total biomass of fish in a small stream. The approach taken in this appendix is the simplest, but not necessarily the most precise. It will be assumed that within the i^{th} selected primary unit a simple random sample of n_i fish is selected and that each of these fish is individually weighed. Individual weight measurements are needed to estimate the variance of the estimated mean fish weight within a selected primary unit.

Methods for estimation of total biomass will depend on the sampling design used, but all methods require estimation of mean fish weight within each selected primary unit. Mean fish weight and variance of mean fish weight within a selected unit can be estimated as:

$$\bar{w}_i = \sum_{j=1}^{n_i} w_{ij}/n_i \text{ ; and}$$

$$\hat{V}(\bar{w}_i) \approx \frac{(1-f_i) \sum_{j}^{n_i} (w_{ij} - \bar{w}_i)^2}{n_i \quad (n_i-1)} ;$$

where:

$\bar{w}_i$ = estimator for mean fish weight in primary unit i;
w_{ij} = weight of the j^{th} fish in the i^{th} unit, $j = 1, 2, \ldots, n_i$
f_i = $n_i/\hat{Y}_i$ = estimated fraction of fish measured in primary unit i; and
$\hat{Y}_i$ = estimated total number of fish in primary unit i.

The above estimator for the variance of the estimated mean weight is approximate because the total number of fish in a primary unit, from which the n_i have been selected, is unknown and must be estimated using some population estimation method.

Case 1.—Primary units selected by simple random sampling: designs A (two-stage SRS) and B (ratio estimation). For these two designs, total biomass can be estimated as:

$$\hat{B} = \hat{Y}\bar{w} ;$$

where: $\hat{B}$ = estimated total biomass of fish in entire stream;
$\hat{Y}$ = estimated total number of fish in entire stream; and
$\bar{w}$ = estimated mean weight of fish in entire stream.

If $\hat{Y}$ and $\bar{w}$ are nearly statistically independent, then variance of $\hat{B}$ can be estimated by (Goodman 1960):

$$\hat{V}(\hat{B}) \approx \bar{w}^2\hat{V}(\hat{Y}) + \hat{Y}^2\hat{V}(\bar{w}) - \hat{V}(\bar{w})\hat{V}(\hat{Y}) .$$

The mean weight of fish in the entire stream, $\bar{w}$, is best estimated as:

$$\bar{w} = \sum^{n} \hat{Y}_i \bar{w}_i / \sum^{n} \hat{Y}_i \ ;$$

where n = total number of selected primary units. This estimator is a weighted average of estimated mean fish weights within selected primary units, where the weighting factors are the estimated numbers of fish in selected primary units.

Because $\bar{w}$ is based on two-stage sampling, with primary units selected by SRS, variance of the overall estimated mean weight can be calculated as (Jessen 1978, p. 291-294):

$$\widehat{MSE}(\bar{w}) \approx \frac{(N-n)}{Nn} \frac{\sum^{n} a_i (\bar{w}_i - \bar{w})^2}{(n-1)} + \frac{1}{Nn} \sum^{n} a_i^2 \hat{V}(\bar{w}_i) \ ;$$

where $a_i = \hat{Y}_i / (\sum^{n} \hat{Y}_i / n)$.

This estimator is similar to the two-stage estimators of variance presented in appendix 2. The first term measures variation among estimated mean weights among selected primary units. The second term measures errors of estimation of mean fish weights within selected primary units. The coefficients, a_i, are adjustments for the number of fish estimated to be present in a particular sampled unit as compared to the average number of fish estimated to be present in a selected primary unit.

Case 2.—Units selected with probabilities proportional to size: design C (PPS without replacement). For this design total biomass within each selected unit is estimated first, and then a two-stage estimator is used to estimate total biomass of fish in the entire stream and the variance of this estimated total. Within any selected primary unit, total biomass can be estimated by:

$$\hat{B}_i = \hat{Y}_i \bar{w}_i \ ;$$

where: $\hat{B}_i$ = estimated total biomass in primary unit i;
$\hat{Y}_i$ = estimated total number of fish in primary unit i; and
$\bar{w}_i$ = estimated mean weight of fish in primary unit i.

$\hat{B}_i$ involves the product of independently estimated quantities, so Goodman's (1960) result can be used to give:

$$\hat{V}(\hat{B}_i) = \hat{Y}_i \hat{V}(\bar{w}_i) + \bar{w}_i \hat{V}(\hat{Y}_i) - \hat{V}(\bar{w}_i)\hat{V}(\hat{Y}_i) \ .$$

Formulas for calculating $\bar{w}_i$ and $\hat{V}(\bar{w}_i)$ are given on the first page of this appendix (appendix 4). $\hat{Y}_i$ and $\hat{V}(\hat{Y}_i)$ would be calculated using formulas appropriate for the particular population estimation method used at the second stage of sampling.

Given estimates of total biomass of fish within each selected primary unit, the total number of fish in the entire stream can be estimated as:

$$\hat{B}_{pps} = \sum^{n} \hat{B}_i/\pi_i \; ;$$

where π_i is the probability that the i^{th} primary unit is in a sample of size n primary units selected by PPS without replacement.

An approximate variance of the estimated total biomass of fish can be calculated by substituting estimated biomass within a selected primary unit for estimated total number of fish in a primary unit and using equation (9) (appendix 2):

$$\hat{V}(\hat{B}_{pps}) \approx \sum_{i}^{n-1} \sum_{j>i}^{n} \frac{(\pi_i\pi_j - \pi_{ij})}{\pi_{ij}} (\hat{B}_i/\pi_i - \hat{B}_j/\pi_j)^2 + \sum^{n} \hat{V}(\hat{B}_i)/\pi_i \; .$$

As for equation (9), the summation is over all distinct pairs of primary units that are in the sample of n primary units.

If the total biomass of fish within selected primary units is highly correlated with the sizes of those primary units, then the PPS design should prove effective for estimating total fish biomass in small streams. The performance of this design for estimating biomass has not been examined, however, and the above formulas should be regarded as preliminary approximations.

Hankin, D.G. Sampling designs for estimating the total number of fish in small streams. Res. Pap. PNW-360. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. **1986.** 33 p.

A common objective of fisheries research is estimating the total number of fish in small streams. The conventional approach involves (1) selecting a small sample of equal-length sections of stream, and (2) estimating the total number of fish in each section using removal method or mark-recapture estimators. Error of estimation of the total number of fish in a stream arises from two sources: (1) extrapolation from the small number of sampled sections to the entire stream, and (2) errors of estimation of fish numbers within sampled sections. This report shows that errors arising from the first source will usually be far larger than those arising from the second source. Total error of estimation can be reduced by making sampled sections equivalent to natural habitat units. Entire pools or riffles should be sampled rather than fixed-length sections of streams. The relative performances of three alternative sampling designs, which can be used when sampled sections are equivalent to natural habitat units, are contrasted in terms of accuracy and cost-effectiveness. Accuracy of estimation can be dramatically improved if sampling designs account for the usually strong, positive correlation between fish numbers and habitat unit sizes.

Keywords: Sampling designs, population sampling, fish population, fish habitat.

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